Geoderma 330 (2018) 1–8

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Elevated [CO_2] changes soil organic matter composition and substrate diversity in an arid ecosystem

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ARTICLE INFO

Handling editor: I. Kögel-Knabner

Keywords:
Soil organic matter
Mass spectrometry
Arid ecosystems
Climate change
CO_2 concentrations

ABSTRACT

Little is known about how elevated atmospheric [CO_2] will impact the dynamics of soil organic matter (SOM) in arid ecosystems. Evans et al. (2014) reported greater ecosystem carbon (C) and nitrogen (N) concentrations following 10 years exposure to elevated atmospheric [CO_2] at the Nevada Desert Free-Air Carbon dioxide Enrichment (FACE) Facility (NDFF). In this study, we investigated potential mechanisms of SOC and total N accumulation and potential SOM stabilization using high resolution mass spectrometry. Samples were collected from soil profiles to 1 m in depth with 0.2 m increment under the dominant evergreen shrub *Larrea tridentate* and were air dried at room temperature. SOM was extracted sequentially with solvents with different polarity. The differences in the molecular composition and diversity of SOM in the different extracts were more evident in surface soils and declined with depth, and were consistent with higher SOC and total N concentrations under elevated than ambient [CO_2]. Our results support the hypothesis that increased root exudation and/or microbial necromass from stabilization of labile C and N can contribute to SOM and N pools. We found that plant-derived compounds were primary substrates for microbial activity under elevated [CO_2] and microbial necromass were the main constituents of stabilized SOM. Our results suggest that arid ecosystems are a potential large C sink under elevated [CO_2], given arid ecosystems constitute 47% of the terrestrial land surface, and that labile compounds are transformed to stable SOM via microbial processes. Arid systems are limited by water, and thus may have a different C storage potential under changing climates than other ecosystems that are limited by nitrogen or phosphorus.

1. Introduction

Arid and semi-arid ecosystems play an important role in the global carbon (C) cycle (Poulter et al., 2014); they are significant components of the terrestrial C budget, represent the fifth largest pool of soil organic C (SOC, 200 pG of C)(Jobbágy and Jackson, 2000) and are under the threat of desertification (Núñez et al., 2009). Although other biomes, such as forests and wetlands, have substantially higher productivity, arid ecosystems can be important contributors to the global C budget with changing climate due to their extensive coverage (47%) in land surface globally (Lal, 2004).

Water-limited ecosystems in arid and semi-arid regions are predicted to be among the most responsive ecosystems to climate change (Whitford, 2002). Elevated [CO_2] could affect arid ecosystems directly by increasing photosynthesis rates and plant productivity, and/or by increasing biotic interactions including nitrogen (N) cycling (de Graaff et al., 2007; Dijkstra et al., 2010), and plant-microbe interactions. Furthermore, elevated [CO_2] could affect arid ecosystems indirectly by changing soil moisture and plant community structure (Fatichi et al., 2016). Increased plant productivity can affect the quality and quantity of organic compounds in the soil (Eskelinen et al., 2009; Reynolds et al., 2003; Wild et al., 2014) through either increased litter input from aboveground (Reynolds et al., 2018) or increased rhizodeposition (de Graaff et al., 2007), thereby shifting substrates available for soil microbial metabolism and ultimately changing soil organic matter (SOM) molecular composition (Eskelinen et al., 2009; Hobbie, 1996).

Organic compounds in soil can be categorized by their lability and decomposability (Smith et al., 2008; Walz et al., 2017; Wild et al., 2016). Labile compounds such as monosaccharides, amino acids and amino sugars are typically rapidly degraded by microorganisms for energy followed by cellulose and lignin, a main component of most plant litter (Fioretto et al., 2005) that are considered less bioavailable (i.e. compounds that need to be degraded by extracellular enzymes before becoming available to microbes). Changes in the ratios of labile

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https://doi.org/10.1016/j.geoderma.2018.05.025
Received 9 January 2018; Received in revised form 15 May 2018; Accepted 23 May 2018
Available online 30 May 2018
0016-7061/ Published by Elsevier B.V.

and recalcitrant compounds will impact soil microbial activities, formation and decomposition of SOM (Hartley and Singh, 2018) and ultimately the C-sink strength of arid ecosystems. Therefore to predict future SOM dynamics in arid ecosystems, it is imperative to understand how the plants and soil microbes will be impacted by elevated [CO_2] (Lipson et al., 2005).

Recently, eddy covariance measurements in arid ecosystems indicated significant C storage but were not supported by field measurements of net primary productivity and were not explained by plausible abiotic mechanisms (Schlesinger, 2017; Schlesinger et al., 2009). How these ecosystems respond to elevated [CO_2] is also understudied relative to mesic ecosystems. In mesic ecosystems without nutrient limitation elevated [CO_2] drives increased biomass production above and belowground (Schlesinger, 2017; Schlesinger et al., 2009).

In this study, we examined the cumulative effects of 10 years of elevated [CO_2] on SOM molecular composition in an intact Mojave Desert ecosystem at the Nevada Desert Free-Air Carbon dioxide Enrichment (FACE) Facility (NDFF). Previously (Evans et al., 2014) reported greater ecosystem carbon (C) and nitrogen (N) concentrations following 10 years exposure to elevated atmospheric [CO_2] at NDFF, due to increased litter input and rhizodeposition in soils. In this study, we investigated SOC and total N accumulation and potential SOM stabilization using high resolution mass spectrometry. Our study objectives include: (i) providing comparative SOM molecular diversity with [CO_2] treatment as well as depth; (ii) assessing indirectly the relative response of microbial communities to litter input and rhizodeposition under elevated [CO_2] to support previous work that has been done at the same site, and (iii) attempting to evaluate the mechanisms behind changes in SOC and total N concentrations under elevated [CO_2] using high resolution mass spectrometry data. High resolution mass spectrometry was the technique of choice and was used to characterize SOM molecular composition as well as assessing indirectly microbial activity and metabolism. We hypothesized that an increase in litter input and rhizodeposition would alter microbial C use and increase the chemical diversity of SOM compounds. We evaluated our hypothesis by measuring SOM molecular diversity to a depth of 1 m at 0.20 m increments under ambient and elevated [CO_2] using high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), an analytical technique capable of providing an in-depth analysis of the molecular composition of SOM. To explore changes in microbial metabolic pathways with elevated [CO_2] compared to ambient [CO_2], we mapped individual SOM compounds onto potential microbial metabolic pathways as inferred from Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/) to reveal the impact that increased litter input and rhizodeposition had on microbial functional potential and metabolism. Our study specifically addresses the potential mechanisms by which elevated [CO_2] impacts SOM composition, substrate diversity and potential microbial activity via changes in organic matter chemical composition and diversity (commonly referred to as organic matter "quality") in an arid ecosystem.

2. Materials and methods

2.1. Study site

The NDFF was established in an undisturbed Mojave Desert ecosystem to examine the response of an arid ecosystem to elevated [CO_2] (Jordan et al., 1999). The climate is arid, with most precipitation occurring as rain during winter months. The site was established in 1997 and consisted of nine 23 m diameter experimental plots, three of which were maintained under elevated [CO_2] (513 μmol mol^{-1}), three additional plots were maintained under ambient [CO_2] (375 μmol mol^{-1}), and three as experimental controls. Treatments began in April 1997 and continued until June 2007. The sparse vegetation at the site is dominated by the evergreen shrub *Larrea tridentata* in addition to other species of deciduous shrubs and perennial grasses. More information about the facility, site, experimental details of the FACE experiment, vegetation, and soils are fully described in Jordan et al. (1999). Prior to [CO_2] treatment, samples were collected in 1997 from center of each plot before [CO_2] treatment commenced. Samples were oven dried (60 °C) and total weight determined. Sample were fractionated using a 2 mm sieve and weights of rock fraction (≥ 2 mm) and soil fraction (< 2 mm) determined. On average samples were 50:50 rocks:soils. Soil samples were further processed through 62.5 μm sieve to remove sand fraction then analyzed for fine soil particle size on Micromeretics Saturn DigiSizer 5200 (Zitzer, 2007). The majority of the particles were > 62.5 μm (> 85%). Coarse silt fraction (between 62.5 μm and 15 μm) represented 6%, followed by fine silt fraction (between 15 μm and 3 μm) and finally the clay fraction represented around 3% (< 3 μm). Soils were categorized as Aridosols with loamy sands in the A1 horizon (0 to 0.16 m) and coarse sands in the deeper horizons (Jordan et al., 1999). Soils were basic with pH ranging from 8 to 9 throughout the depth to 1 m (Jordan et al., 1999). Bulk density varied from 1.43 (0 to 0.2 m) to 1.31 (0.6 to 0.8 m) (*unpublished data*).

Following [CO_2] treatment, samples were collected between July and October 2007 belowground in triplicates from the ambient and elevated [CO_2] plots in 0.2 m increments between 0 and 1 m (total of 5 samples per depth profile) under the dominate vegetation shrub *Larrea tridentata* after ten years of exposure to elevated [CO_2]. Upon collection, soil samples were air-dried in polyethylene bags at ambient temperature in a storage shelter at NDFF. In November 2007, soil samples were transported to Washington State University, Pullman, Washington, USA, where soil samples were sieved through 2 mm screen, and stored in polyethylene bags at room temperature. Soil samples from each depth and each treatment (N = 3 for ambient and N = 3 for elevated) were then pooled to be analyzed by high resolution mass spectrometry at the Environmental Molecular Sciences Laboratory in Richland, WA. Since % SOC and % N values (Fig. 1) showed slight variations between plot replicates, pooling the biological replicates minimizes the effect of soil heterogeneity at the experimental plot scale.

Soil C and N contents were determined using an ECS 4010 elemental analyzer (Costech Analytical, Valencia) interfaced with an isotope ratio mass spectrometer (Delta PlusXP, Thermo Finnigan) at the Stable Isotope Core facility, Washington State University, Pullman, Washington, USA, after carbonate removal. The data were reported in per gram (g) of dry solids (d.s). Throughout the manuscript, the depth increment 0–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, 0.8–1 m is denoted by the average depth for that increment (0.1, 0.3, 0.5, 0.7 and 0.9 m) respectively.

2.2. Soil organic matter extraction

Three solvents (water, methanol and chloroform) with different polarities were used to sequentially extract a representative fraction of organic matter from belowground soils according to protocol 1 in Tfaily et al. (2017). Briefly, extracts were prepared by adding 1 mL of solvent to 100 mg bulk soil and shaking in 2 mL capped glass vials for 2 h on an Eppendorf Thermomixer. Samples were removed from the shaker and left to stand before spinning down and pulling off the supernatant to stop the extraction. The soil residue was dried with nitrogen gas to remove any residual solvent, and then the second solvent (e.g., MeOH, or $CHCl_3$) was added and so on. The $CHCl_3$ and H_2O extracts were diluted in MeOH to improve ESI efficiency. We found that $CHCl_3$ extractions required the elimination of all plastic sample-handling materials to prevent the introduction of poly ethylene glycol (PEG) into the extracts which adversely affects the ionization the organic matter compounds extracted from the soil. Depending on the soil and mineralogy, this protocol can extract anywhere from 1 to 7% of the total soil C (Tfaily et al., 2017). This protocol targets free or loosely bound organic matter compounds i.e., organic matter that is easily accessible for microbial communities rather large biopolymers or mineral-bound compounds. Even though we are only extracting < 7% of total C pool,

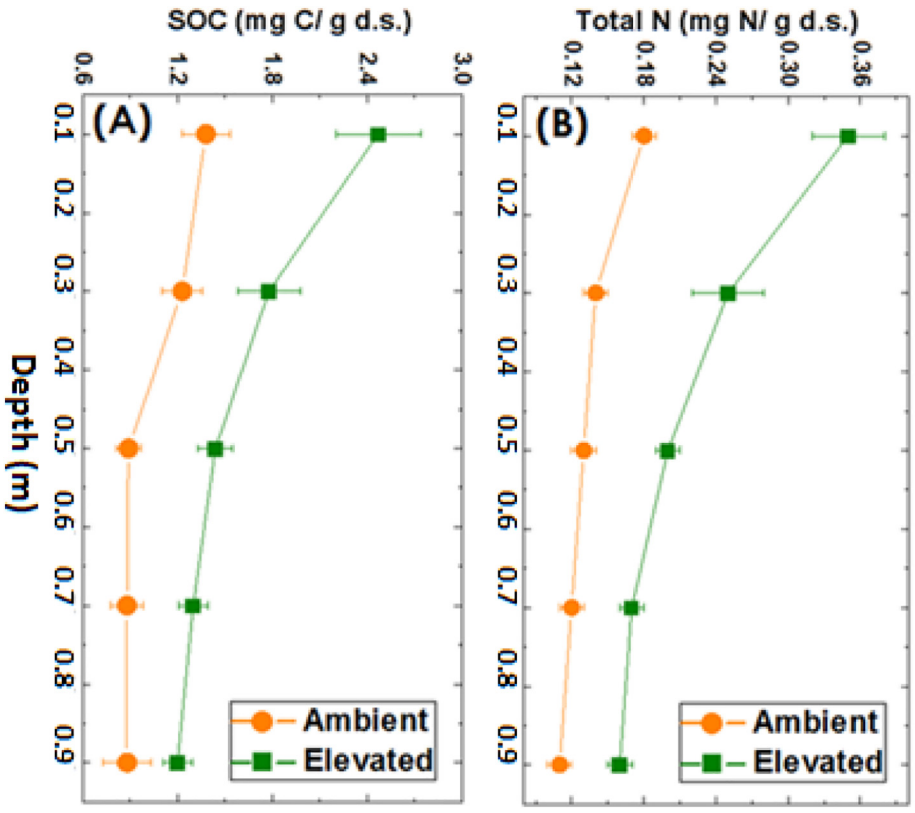

Fig. 1. Depth profile of (A) soil organic carbon concentrations in mg C/g d.s and (B) total N concentrations in mg N/g d.s under ambient (orange circles) and elevated (green squares) CO_2 concentrations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

we believe that this protocol samples the substrates that drive microbial activity in soils, including the mobile and available portion of the total organic matter pool known as Water-Extractable Organic Matter (WEOM) (Corvasce et al., 2006). WEOM is defined as the soluble fraction of organic matter extracted from the soil under various laboratory conditions, the most active and mobile fraction of soil organic matter (SOM). Although WEOM is only a small part of soil organic matter, it has been shown to have a strong influence on several ecologically relevant processes in soil. It is a potential C source for soil microorganisms, modulating soil microbial community via the changes of quality and quantity of C compounds and their bioavailability (Bausenwein et al., 2008; Embacher et al., 2007; Kalbitz et al., 2000). Methanol and chloroform extractions are then used to extract the rest of the semi-polar and non-polar compounds that are free or loosely bound to mineral surfaces.

2.3. FTICR data acquisition and data analysis

A 12 Tesla Bruker SolariX FTICR spectrometer located at EMSL, a DOE-BER national user facility located in Richland, WA, was used to collect high resolution mass spectra of the OM in the extracts. A standard Bruker ESI source was used to generate negatively charged molecular ions. Samples were then introduced directly to the ESI source. The instrument was externally calibrated weekly to a mass accuracy of < 0.1 ppm using a tuning solution from Agilent, which contains the following compounds: $C_2F_3O_2$, $C_6HF_9N_3O$, $C_{12}HF_{21}N_3O$, $C_{20}H_{18}F_{27}N_3O_8P_3$, and $C_{26}H_{18}F_{39}N_3O_8P_3$ with an m/z ranging between 112 and 1333. The instrument settings were optimized by tuning on a Suwannee River Fulvic Acid (SRFA) standard. Blanks (HPLC grade MeOH) were also ran at the beginning and the end of the day to monitor potential carry over from one sample to another. The instrument was flushed between samples using a mixture of water and methanol. The ion accumulation time (IAT) was varied to account for differences in C concentration between samples and varied between 0.1 and 0.3 s. Ninety-six individual scans were averaged for each sample and internally calibrated using OM homologous series separated by 14 Da ($-CH_2$ groups). The mass measurement accuracy was < 1 ppm for singly charged ions across a broad m/z range (i.e. 200 < m/z < 1200). To further reduce cumulative errors, all sample peak lists for the entire dataset were aligned to each other prior to formula assignment to eliminate possible mass shifts that would impact formula assignment. Putative chemical formulas were assigned using Formularity software (Tolić et al., 2017). Chemical formulas were assigned based on the following criteria: S/N > 7, and mass measurement error < 1 ppm, taking into consideration the presence of C, H, O, N, S and P and excluding other elements. Peaks with large mass ratios (m/z values > 500 Da) often have multiple possible candidate formulas. These peaks were assigned formulas through propagation of CH_2, O, and H_2 homologous series. Additionally, to ensure consistent choice of molecular formula when multiple formula candidates are found the following rules were implemented: we consistently pick the formula with the lowest error with the lowest number of heteroatoms and the assignment of one phosphorus atom requires the presence of at least four oxygen atoms. Peaks that were present in the blanks were subtracted from the sample data sets. Additionally, all single peaks i.e. peaks that are present in only one sample were removed and are not included in the downstream analysis. To investigate the compositional changes in SOM with the [CO_2] treatment and depth, we calculated a composite composition by combining the common compounds that were observed in spectra produced by the three solvents (i.e., H_2O, MeOH, and $CHCl_3$) and the compounds that were uniquely extracted by each of the three solvents. Total number of peaks ranged between 5000 and 10,000 peaks for the ambient plots compared to 8000–13,000 for the elevated plots. The percentage of compounds assigned with a molecular formula ranged between 38 and 47% for the ambient plots compared to 39–52% for the elevated plots.

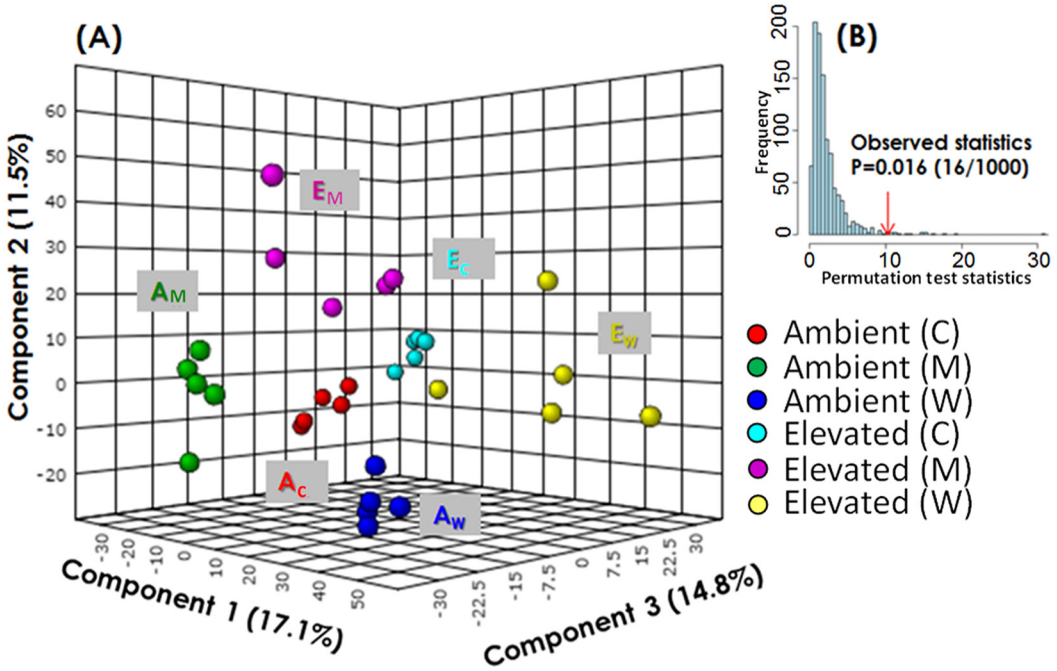

Fig. 2. (A) A PLS-DA 3D scores plot between the first three PCs. The explained variances are shown in brackets; (B) PLS-DA model validation by permutation tests (1000 permutations) based on separation distance. The P value based on permutation is P = 0.016 (16/1000). "C" refers to chloroform; "M" refers to methanol; and w: refers to water.

To visualize differences in SOM composition, compounds that were unique to each sample were plotted on a van Krevelen diagram corresponding to their H/C (hydrogen to carbon) vs. O/C (oxygen to carbon) ratios (Kim et al., 2003). Van Krevelen diagrams provide a means to visualize and compare the average properties of OM and assign compounds to the major biochemical classes (i.e., lipid-, protein-, lignin-, carbohydrate-, Carboxylic-Rich Alicyclic Molecules (CRAM)- and condensed aromatic-like) (Kim et al., 2003). In this study, labile compounds refer to compounds that are readily utilized by microbial communities such as sugars and proteins, while the recalcitrant fraction is utilized with the aid of extracellular enzyme such as lignin (Brookshire et al., 2005). Venn diagrams were used to distinguish between shared and non-shared (unique) formulas observed between ambient and elevated [CO_2] for the surface (0.1 m) and deep (0.9 m) samples. Home built software was then used to match the putative formulas to identified KEGG metabolic pathways to suggest the potential pathways present in our study. As such, the number of metabolites involved in each specific pathway was counted and compared between samples. Principle component analysis was performed using MetaboAnalyst (Xia et al., 2009, 2012, 2015; Xia and Wishart, 2002a, b). For MetaboAnalyst, FTICR-MS data were first normalized by the median, log transformed and scaled by Pareto scaling (mean-centered and divided by the square root of standard deviation of each variable) before the plots were generated (PCA plots, PLS-DA plots and heat maps). The PLS-DA model was validated by permutation test statistics at 1000 permutations with observed statistics P = 0.016. For PCA analysis by OriginPro, the relative abundance of the different classes of compounds (CHO, CHON, CHOS, etc.) identified by FTICR-MS were utilized along with biogeochemical data (%C. %N, Total N, C:N ratios, $d^{13}C$ and $d^{15}N$). A home built program was then used to calculate the mass difference between *m/z* peaks extracted from each spectrum with S/N > 7 within 1 ppm.

We used molecular transformation analysis to identify potential microbial transformation or decomposition pathways, the mass difference between m/z peaks extracted from each spectrum with signal-to-noise ratio > 7 were compared to commonly observed mass difference for common metabolic reactions (Breitling et al., 2006; Longnecker and Kujawinski, 2016). In this way, a mass difference of 2.0156 Da represents a hydrogenation/dehydrogenation reaction whereas a mass difference of 71.0371 Da represent a reaction where an alanine molecule (C_3H_5NO) has been lost or gained, and so on. Chemically transformed species will be related by measurable, clearly defined mass differences, which are known for common metabolic reactions (Breitling et al., 2006; Longnecker and Kujawinski, 2016) and can be uniquely measured by ultra-high resolution FTICR-MS. We then counted how often a particular mass difference (and thus a biochemical reaction) occurred for each sample. The results were then visualized using Principle Component Analysis.

3. Results and discussion

3.1. Bulk SOC and total N concentrations

Bulk SOC and total N concentrations were significantly greater under elevated [CO_2] than ambient [CO_2] (Fig. 1). Moreover, this enhancement was more evident with soils closer to the surface (0.1 m) compared to the deeper soils (0.9 m). Three primary causes were hypothesized to be responsible for the observed increase, all of which are tied to enhanced photosynthetic performance and plant water status in *Larrea* under elevated [CO_2] that was observed in previous work (Evans et al., 2014; Jin and Evans, 2010; Koyama et al., 2018; Weatherly et al., 2003): (i) increased litter input from above ground; (ii) increased rhizodeposition; and (iii) subsequent assimilation and formation of SOM by the soil microbial communities. These hypotheses were also consistent with that isotopic mass balance calculations by Evans et al. (2014) that suggested that in the Mojave Desert, around 70% of the C that accumulated belowground originated from the recent litter input, compared to around 30% that originated from sources belowground such as rhizodeposition and microbial activity.

3.2. Organic matter composition and microbial activity by FTICR-MS

The normalized relative intensities of the measured mass spectrometry peaks were utilized in Partial Least Squares-Discriminant analysis

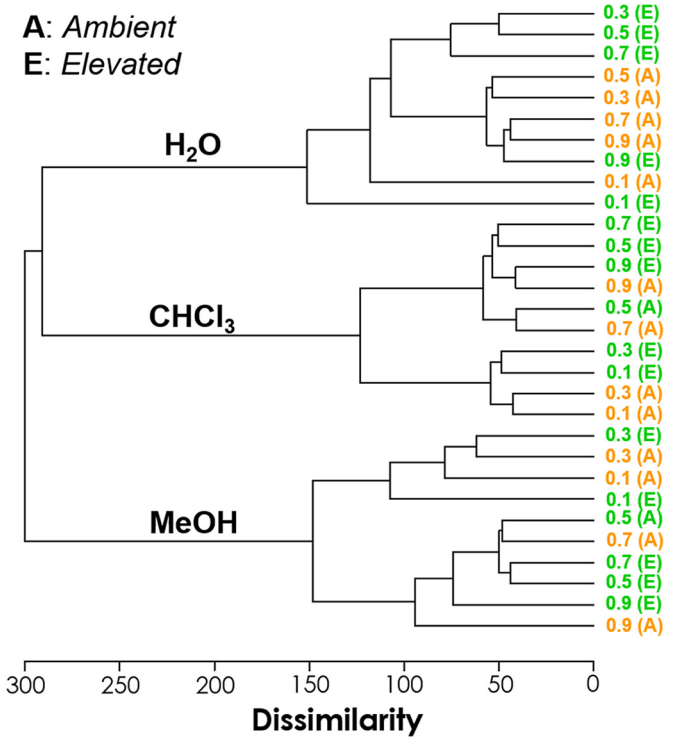

Fig. 3. Hierarchical cluster dendrogram of the molecular composition from all soil samples under ambient and elevated [CO_2] with depth and per solvent type.

(PLS-DA) plots (Fig. 2). FTICR-MS measurements revealed significant SOM compositional differences ($P < 0.05$) between the ambient and elevated [CO_2] soils along the depth profiles, for each of the solvents used (Fig. 2). Two distinct groups can be seen for each solvent type, corresponding to ambient or elevated [CO_2] treatment ($P = 0.016$) (Figs. 2–3) suggesting that each solvent extracted a unique set of compounds depending on its polarity. Additionally, for each solvent type, SOM composition for the surface soils (0–0.5 m) was different than the deeper samples (0.5–1 m) (Fig. 3).

SOM compositional differences were clear between ambient and elevated [CO_2] for the surface samples (Fig. 4, upper panel) and appeared to be shaped in part by the increased litter input and rhizodeposition under [CO_2] enhancement (discussed below). In particular, > 50% of the compounds present in the elevated [CO_2] at 0.1 m were unique, and absent in the corresponding sample under ambient [CO_2] (Fig. 4). Distinct compound groups can be seen for elevated [CO_2] at the surface (0.1 m): sugar-like and protein-like compounds that represent labile compounds (readily utilized) and lignin-like and CRAM-like compounds most likely originating from surface foliage; whereas the ambient [CO_2] soils at the surface were more dominated by lipid-like compounds characterized by low O/C and high H/C ratios with little to no labile compounds (Fig. 4). The increase in the abundance of labile compounds under elevated than ambient [CO_2] at the surface therefore indicates that elevated [CO_2] increased root exudates and/or litter deposition consisting of labile compounds such as organic acids. A decrease in the abundance of labile compounds was observed with depth for the elevated [CO_2] samples with an increase in the abundance of lipid-like compounds. Because microbial biomass includes abundant lipid-like compounds, and compounds with low O/C ratios and relatively high H/C ratios (Tfaily et al., 2013), the lipid-like compounds in the deep samples under elevated [CO_2] are likely of microbial origin (i.e. microbial necromass). Additionally, previous work have shown that leaf and root litter decomposition products, mainly microbial residues produced during litter degradation, are known to contribute a large amount of C to the mineral soil (Bird et al., 2008; Rubino et al., 2010). Field observations in the Mojave Desert have suggested increased mineral-bound soil organic C under elevated [CO_2], consistent with increased rhizodeposition and or litter deposition (Nowak et al., 2012). This observation was consistent with the increase in microbial residue and microbial-derived compounds (lipid-like compounds with depth) (Cotrufo et al., 2013; Mambelli et al., 2011; Sollins et al., 2009) under elevated [CO_2] compared to ambient [CO_2] especially at depth (Fig. 4B-lower panel). Increased microbial residues in elevated [CO_2] samples was also supported by microbial lipid analysis where elevated [CO_2] doubled bacterial neutral lipid fatty acids (NLFA) originating from bacterial necromass (Koyama et al., 2018). The increase in rhizodeposition and litter input (described above) did not result in utilization of previously stored SOM. In this arid ecosystem and under elevated [CO_2], soil microorganisms appear to shift from consuming older recalcitrant C to utilizing easily degraded labile compounds derived from increased litter input and rhizodeposition. Soil microbes at this site are known to be C and thus energy limited (Jin et al., 2011) and increased C assimilation by plants under elevated [CO_2] as previously shown by Evans et al. (2014) ultimately increased C supply to soil microbes through rhizodeposition and/or root exudation.

To gain further insight of the impact of elevated [CO_2] on microbial functional potential and metabolism, assessed indirectly by high resolution mass spectrometry, and to confirm our above hypothesis we mapped the unique compounds observed under each treatment (ambient and elevated [CO_2]) onto microbial metabolic pathways (Fig. 5) as inferred from KEGG database (http://www.genome.jp/kegg/). In particular, for surface samples under elevated [CO_2], we observed an increase in amino acid, carbohydrate and polyketides metabolism in response to increased input from surface vegetation, and compared to ambient samples that were mainly undergoing lipid metabolism (Fig. 5A). The impact of elevated [CO_2] was still detectable at depth for the elevated [CO_2] soils (Fig. 5B) but to a lesser extent compared to the surface. This was evident in the decrease of amino acid, carbohydrate and polyketides metabolism for the elevated [CO_2] soils with depth and an increase in lipid metabolism. Limited potential microbial activity was inferred with depth for the ambient CO_2 soils (Fig. 5B). These data therefore may suggest that the dominant microbial decomposition pathways observed in this study, involved labile substrates potentially provided from rhizodeposition and recent litter input (Fig. 5) as their energy source. The increase in terpenoids and polyketides metabolism on the other hand may reflect increased production or biosynthesis of secondary metabolites by living organisms in order to impart to them some survival advantage, in this case, elevated CO_2 concentration.

3.3. Dynamics of N-containing compounds

PCA analysis of the relative abundance of the different classes of compounds such as N-containing compounds, S-containing compounds and biogeochemical properties (Fig. 6) revealed an increase in the abundance of N-containing compounds under elevated [CO_2], consistent with an increase in total N concentration under elevated [CO_2] (Fig. 1B). The increase in the abundance of N compounds was accompanied by the increase of the abundance of amino-sugars, lignin/CRAM and proteins and sugar-like compounds at the surface (Fig. S1A) and lipid-like compounds with depth (Fig. S1B). These data therefore may suggest that above- and belowground inputs increased litter input from aboveground and rhizodeposition belowground provided labile compounds characterized by high N concentrations (Fig. 1B) that may have acted as high-quality substrates for microbes (Novara et al., 2015).

Furthermore, molecular transformation analysis revealed an increase of amino acid-associated transformations in the elevated [CO_2] soils relative to ambient [CO_2] (Fig. S2) with a clear transition from N-free transformations in ambient [CO_2] soils to N-containing transformations in the elevated [CO_2] soils (Fig. S1). Studies have shown that substrates rich in N can accelerate the formation and accumulation of

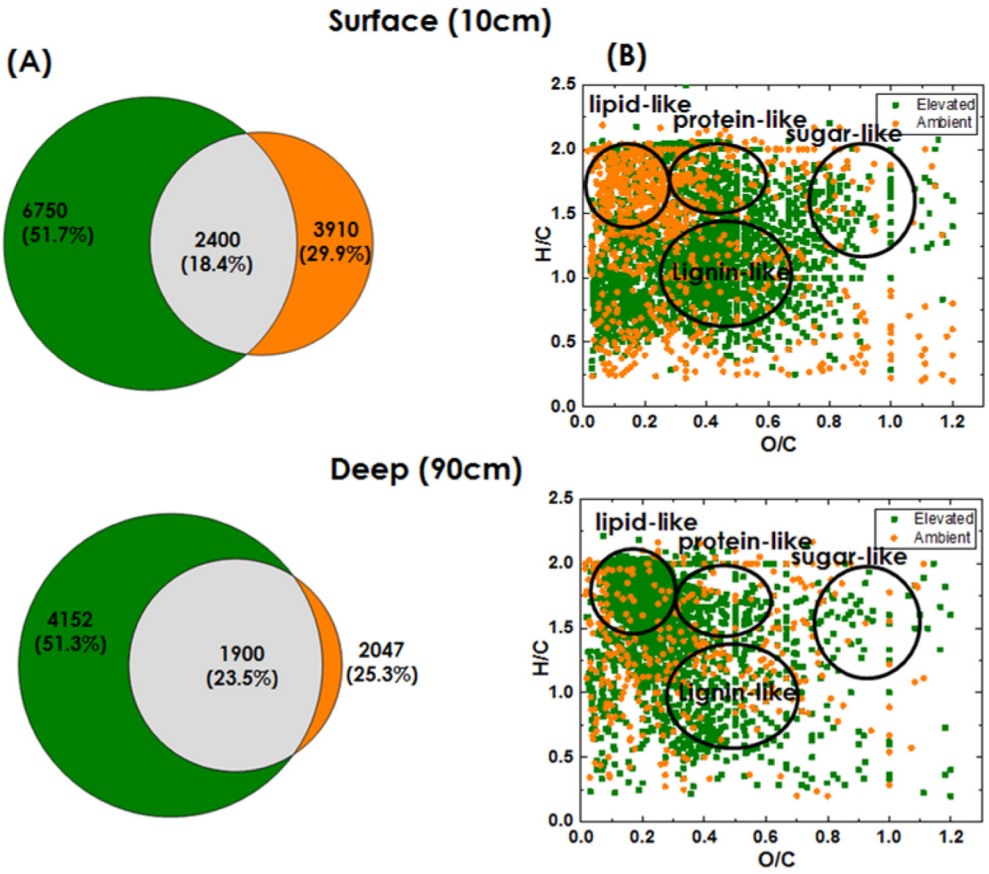

Fig. 4. (A) Venn diagram and (B) van Krevelen diagram comparing the molecular composition under ambient (orange circles) and elevated (green squares) CO_2 concentrations for the 0.1 m samples (upper panel) and the 0.9 m sample (lower panel). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microbial residues (Liang et al., 2015). Figs. 6 and S2 showed that samples under elevated [CO_2] are rich in N. This, in turn, may enable microbes to overcome N limitation (if present), thereby allowing a more efficient use of C for microbial biomass growth (Kaiser et al., 2015). However, previous results from this experiment and direct C additions (Schaeffer et al., 2013) have shown that in this system, microbial

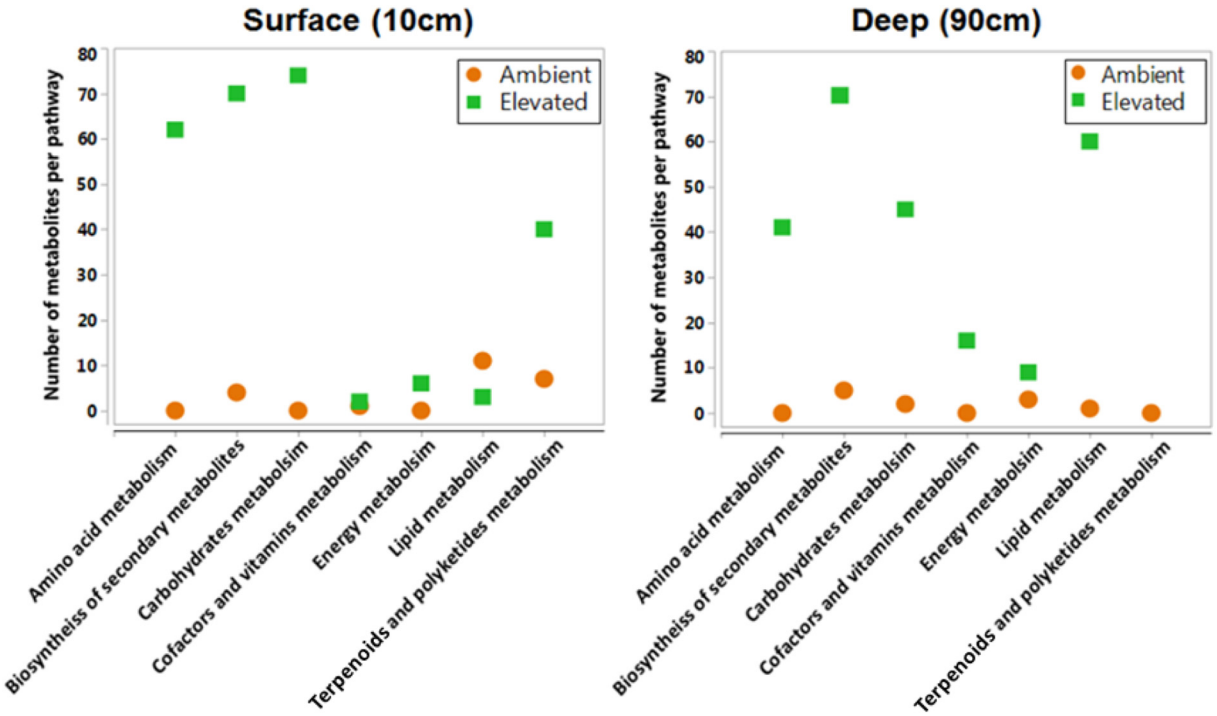

Fig. 5. Potential microbial pathways utilized in organic matter degradation and transformation under ambient (orange circles) and elevated (green squares) CO_2 concentrations for the (A) 0.1 m and (B) 0.9 m samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

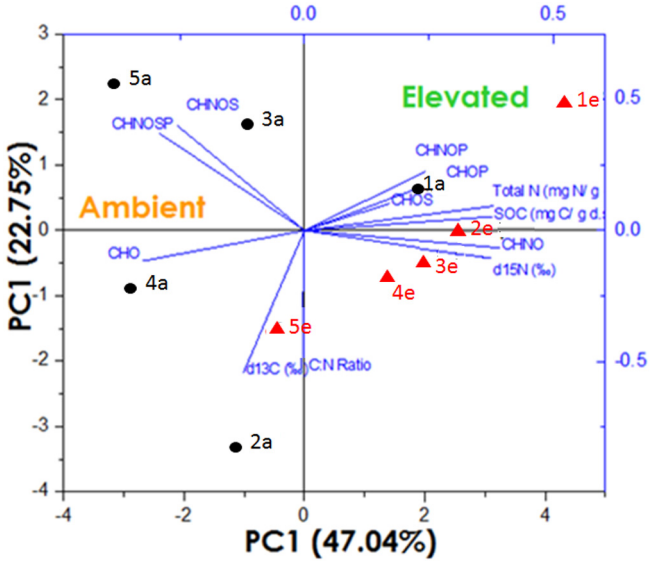

Fig. 6. Principal components analyses (PCA) of FT-ICR-MS elemental composition classes and biogeochemical properties. Scores for each sample are shown as points, and variable loadings for each PC are shown as labeled arrows. "a": represents ambient and "e" represents elevated. "1" reflects samples at 0.1 m, "2" 0.3 m, "3" 0.5 m, "4" 0.7 m and "5" 0.9 m.

activity is actually limited by C and not N. We therefore hypothesized that in this system, elevated [CO_2] stimulated plant growth which in return increased C supply to soil microbes. Greater inputs of C to soils will then stimulate soil microbial activities; and this enhanced microbial activity would increase plant-available N allowing plants to produce more litter and/or root exudates. The observed increase in microbial activity and fungal biomass due to this labile source of C could increase rates of N cycling and transformation belowground (Billings et al., 2004; Jin and Evans, 2007; Jin et al., 2011). The observed increase in the amino acid-associated transformations in the elevated [CO_2] relative to the ambient [CO_2] soils (Fig. S2 and Fig. 5) confirm this hypothesis and further suggest microbial assimilation and stabilization of organic compounds from recent litter input and rhizodeposition into microbial residues. Recently Koyama et al. (2018) showed that fungal NLA were 2.4 times higher in elevated than ambient soils suggesting that fungi accumulated NLFA as storage compounds under elevated [CO_2]. We therefore hypothesize that when provided with labile compounds, such as root exudates, and with limited supply of nutrients, fungi assimilate the excess labile C and store it as NLFA (Bååth, 2003; Koyama et al., 2018) likely contributing to increased total N concentrations and transformations under elevated [CO_2]. After C and N-containing substrates are assimilated into microbial residues, their fate is dependent on many factors, including chemical protection, physical protection and soil matrix interaction. In our system we believe that SOM with depth is mainly protected from further decomposition under elevated [CO_2] due to its inherent recalcitrance (i.e., abundance in alkyl groups) (Dungait et al., 2012; Tfaily et al., 2013) however matrix stabilization by silt and clay particles can further contribute to SOM stabilization with depth (Nowak et al., 2012).

4. Conclusions

Our data suggested that arid ecosystems under elevated [CO_2] and continued water limitation results in increased microbial biomass stimulated both by rhizodeposition and aboveground litter. We found that plant-derived compounds were primary substrates for microbial activity under elevated [CO_2] and microbial necromass were the main constituents of stabilized SOM Coordinated shifts in dominant organic C transformations, dominant organic C compounds (inferred from van Krevelen analyses, see Figs. 4 and S1), and microbial metabolic pathways (inferred from KEGG) suggested significant connections among organic C chemistry, and microbial activity in this arid ecosystem. This contrasts with mesic forest ecosystems where increasing plant litter inputs did not increase soil C but instead, enhanced SOM degradation, suggesting that different ecosystems may exhibit varying responses to future environmental changes that lead to increased plant productivity and plant inputs to soils (Castellano et al., 2015; Lajtha et al., 2014). Our results suggest that arid ecosystems might present a unique opportunity to study SOM accumulation where microbial necromass represent a significant component of its composition. Arid systems are limited by water, and thus may have a different C storage potential under future elevated [CO_2] than other ecosystems that are limited by nitrogen or phosphorus.

Acknowledgments

FT-ICR-MS analysis was performed at EMSL, a DOE Office of Science User Facility sponsored by the Office of Biological and Environmental Science, Climate and Environmental Sciences Division, The Terrestrial Ecosystem Science (TES) program (FWP 65324) and located at Pacific Northwest National Laboratory. MMT thanks DOE-BER TES for financial support. We would like to thank the reviewers for their thoughtful comments and efforts towards improving our manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2018.05.025.

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